Morphological changes and the associated fungal colonization during decomposition of leaves of a mangrove, *Bruguiera gymnorrhiza* (Rhizophoraceae)

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Leaves of *Bruguiera gymnorrhiza* (L.) Lam. growing at Beachwood Mangrove Nature Reserve (Durban) were removed just prior to leaf fall, placed in mesh litterbags and submerged in the creek of the swamp. At the time of collection the leaves were colonized by phylloplane fungi. The degree of decomposition of leaf material was studied over a period of 18 weeks by means of SEM and LM techniques. After a week's submergence, the leaves appeared to be devoid of phenolic deposits and microbial colonization intensified. SEM studies indicated that fungi may have degraded the cuticular waxes but the cuticle was still intact at the end of the study. Degradation of the internal tissues was shown to be associated with fungal activities.

Blare van *Bruguiera gymnorrhiza* (L.) Lam. wat in die Beachwood Mangliet-natuurreservaat (Durban) groei, is net voor blaarval verwyder, in maasafvalsakke geplaas en in die inham van die moeras onder water gelaat. Toe die materiaal versamel is, het blaarlewende fungusse daarop voorgekom. Die graad van ontbinding van blaarmateriaal is oor 'n tydperk van 18 weke deur middel van aftaselektronmikroskoop- en ligmikroskooptegnieke bestudeer. Na 'n week in die water was daar skynbaar geen fenoliese verbindings meer in die blare nie en het mikrobiese kolonisering toegeneem. Ondersoeke met die aftaselektronmikroskoop het getoon dat die kutikulêre waslae moontlik deur fungusse afgebreek is maar dat die kutikula aan die einde van die studie steeds heel was. Daar is getoon dat die afbraak van die interne weefsels met fungusaktiwiteit geassosieer is.

Keywords: Cuticle, cuticular perforations, degradation, epicuticular waxes, mangrove

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Introduction

Few reports showing the patterns of mangrove leaf decay as illustrated by scanning electron microscopy (SEM) exist in the literature. The lack of information on the degradation of epicuticular wax prompted MacNamara and Dickinson (1981) to recommend SEM as a useful tool for studying its destruction. Fungi have been shown to be involved in decomposition of plant litter in terrestrial ecosystems (Swift *et al.* 1979), freshwater (Kaushik & Hynes 1971), seawater (Anastasiou & Churchland 1969) and in mangrove swamps (Fell & Master 1973). Cundell *et al.* (1979) examined the surfaces of leaves of *Rhizophora mangle* L. and showed a diversity of micro-organisms on them after 35 days of submergence in seawater.

Previous articles by Steinke *et al.* (1983) and Steinke and Charles (1986) lack details of the microbial contribution to mangrove litter decomposition. The present study contributes towards making up for this deficiency.

Materials and Methods

The study was conducted in the Beachwood Mangrove Nature Reserve at the mouth of the Mgeni River, Durban (29°48'S; 31°02'E). Raiman (1986) has provided a detailed account of the vegetation of the reserve. Briefly, the mudflat vegetation is dominated by mangroves with *Bruguiera gymnorrhiza* (L.) Lam. and *Avicennia marina* (Forssk.) Vierh. being the dominant trees, although *Rhizophora mucronata* Lam. does occur. *Juncus kraussii* Hochst. and *Stenotaphrum secundatum* (Watt) Kuntze, typical salt marsh plants, occur in the drier areas.

The Beachwood Creek flows southwards through the swamp for about 3 km. Freshwater flows into the creek and diurnal tidal variation results in the inflow of saline waters. Salinities of above 30‰ were recorded up to 1410 m from the mouth of the creek at high tide (Raiman 1986).

Leaves were hand-picked from trees of *B. gymnorrhiza*. The leaves collected were yellow, close to abscission and detached easily from the parent tree. In the laboratory they were divided into 88 groups, each of approximately 21 leaves with a fresh mass of 49.95 g ± 0.2 g. Each weighed group was placed in a nylon mesh bag (25.5 × 22 cm), mesh size 2.5 × 5 mm. Bags were ruched closed with fishing line which was threaded through a hole in one of three wooden stakes each carrying 26, 26 and 28 bags respectively. The bags were arranged to prevent overlapping. The next morning the stakes were hammered into the mud at the research site in the creek to a height of about 10 cm, so that bags were submerged even during low tide.

Bags were collected on weeks 0, 1, 2, 3, 5, 7, 9, 11, 13, 16 and 18. At each collection, eight litterbags were taken to the laboratory within 45 min of collection, in a sterile plastic bag. The combined contents of four of the bags were repeatedly washed in a gentle stream of tap water.

The surfaces of young, mature and senescent leaves of *B. gymnorrhiza* (still attached to parent trees) were examined on a monthly basis. Fresh leaves were hand-cut and stained with safranin and fast green for light microscopy. They were

also stained for 5 min in ferric chloride (2% in 95% ethanol), to test for phenolics (Gahan 1984).

Samples of washed leaf material, submerged (from litterbags) and pre-abscissed (young, mature and senescent), were rapidly frozen in liquid nitrogen, fragmented and then dried in an Edwards Modulyo freeze dryer at –40 to –60°C at a vacuum pressure of 400 Pa for 18 h. Selected leaf fragments, secured onto brass stubs with double-sided sellotape, were coated with gold in an atmosphere of argon in a Polaron Sputter coating unit E 5000, at a voltage of 1.1 kV for 5 min. Coated material was examined and photographed in a Philips SEM 500 at 12 kV at 32 lines per second on Pan F film.

Results

SEM of leaf surfaces

Pre-abscissed leaves

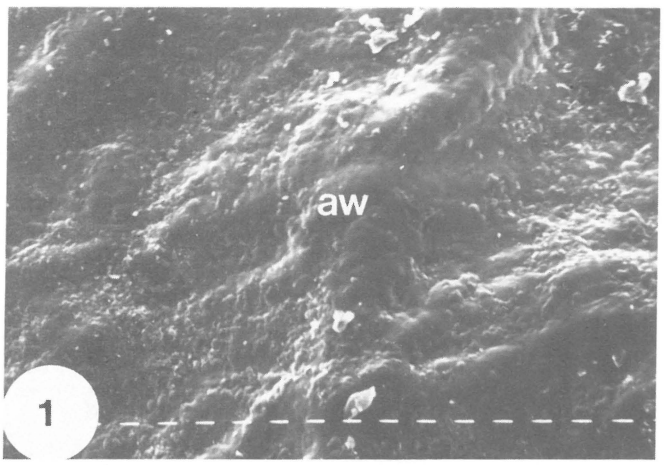
The adaxial surface of young leaves was covered with a layer of amorphous wax (Figure 1). As the leaf matured, crystalline wax (Baker 1982) in the form of rounded bodies and plates was superimposed on the amorphous layer. Phylloplane fungi were not observed.

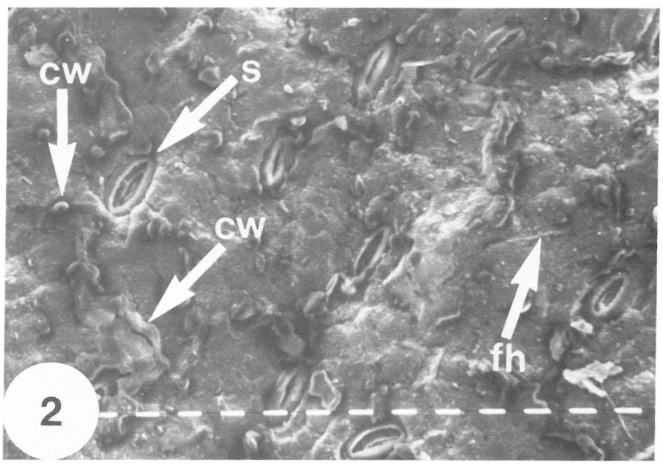
Stomata were only present on the abaxial surface. Amorphous and crystalline wax as well as traces of fungal hyphae were seen on the lower surface of yellow senescent leaves (Figure 2).

Submerged leaves (litterbags)

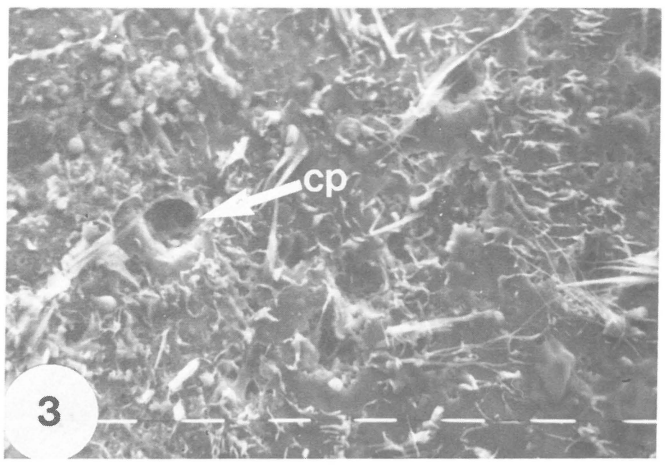
After one week of submergence the upper leaf surface was characterized by the formation of cuticular perforations. These perforations appeared to be breakdown points at which the cuticle was eroded, thereby exposing the underlying tissue. The perforations were generally eucentric in shape, although irregular shapes were also observed. Most of the crystalline wax had disappeared and fungal growth on the amorphous wax was evident (Figure 3).

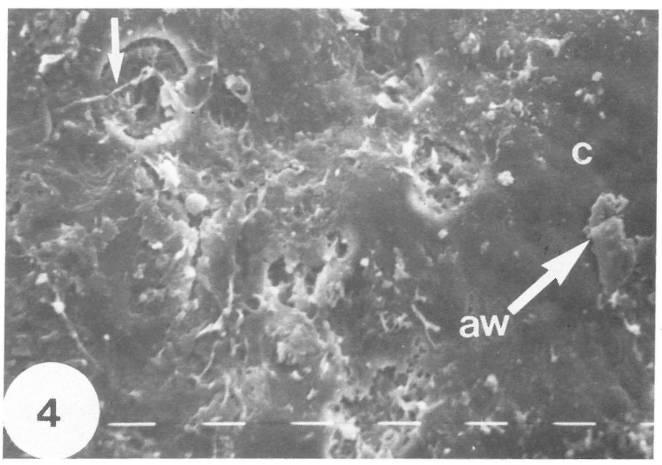
After three weeks, the upper leaf surface was heavily colonized with fungal mycelia. After five weeks, cuticular perforations were more clearly observed and fragments of wax were scattered on the leaf surface. The perforations were penetrated by fungal hyphae and may have provided a



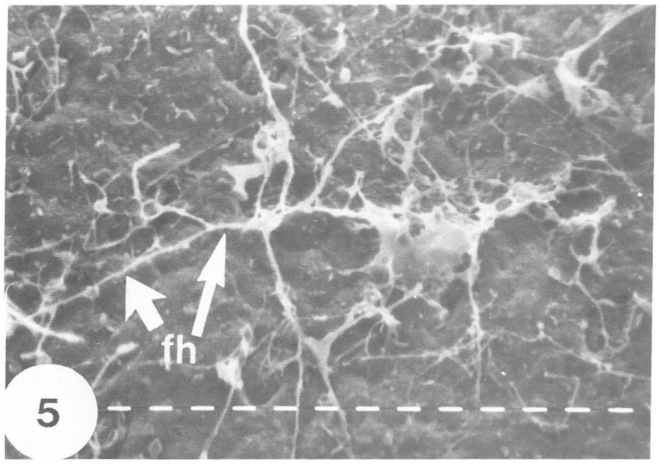


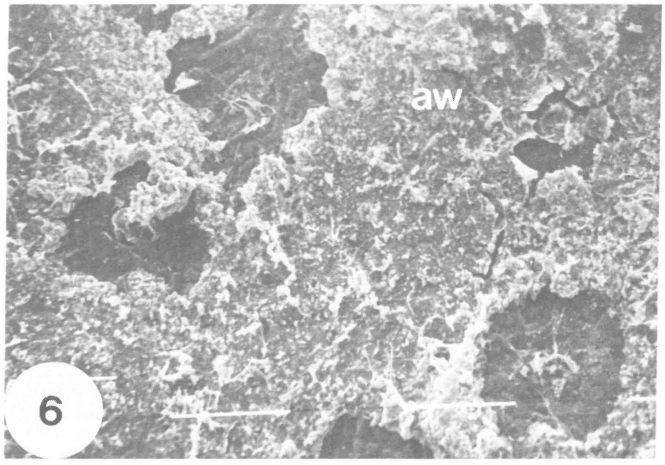
Figures 1 – 2 Scanning electron micrographs of pre-abscissed leaf surfaces of *B. gymnorrhiza*. 1. Adaxial surface of young green leaf showing amorphous nature of the cuticular wax (aw, amorphous wax). 2. Abaxial surface of yellow senescing leaf with stomata (s), crystalline wax (cw) and fungal hypha (fh). Bars = 10 μm.

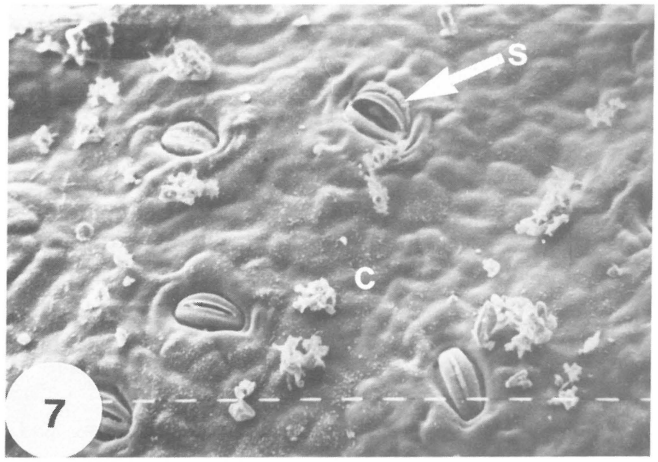




Figures 3 – 4 Scanning electron micrographs of the adaxial surface of decomposing leaves of *B. gymnorrhiza*. 3. After one week, showing cuticular perforations (cp) and fungal growth. 4. After five weeks, showing scattered fragments of amorphous wax (aw) on cuticle (c) and cuticular perforations penetrated by fungal hypha (arrow). Bars = 10 μm.







Figures 5 – 7 Scanning electron micrographs of the abaxial surface of decomposing leaves of *B. gymnorrhiza*. 5. After one week traversed with fungal hyphae (fh). 6. Amorphous wax (aw) breakdown after three weeks. 7. Unaltered structure of stomata (s) and cuticle (c) at the end of the study period. Bars = 10 μm in Figures 5 and 7 and 100 μm in Figure 6.

passage-way for fungi into the internal tissues of the leaf (Figure 4). In addition to wax breakdown there was a reduction in the growth of the surface fungi. At the end of the study, the cuticle was still intact.

After one week of submergence, the epicuticular wax on the abaxial surface was generally amorphous, suggesting early breakdown of crystalline wax. Stomatal pores were often traversed by fungal hyphae and may have provided access to inner tissues (Figure 5).

After three weeks, fungal colonization was intense and amorphous wax breakdown was evident (Figure 6). Wax breakdown was more pronounced around a stoma but this phenomenon became less evident as breakdown progressed. After 11 weeks most of the epicuticular wax was degraded and the cuticle was exposed. Except for the disappearance of their epicuticular waxes, the surface structure of the stomata was unaltered during the period of decomposition (Figure 7).

Internal structure

Pre-abscissed leaves

Intracellular phenolic deposits which yielded a green colour in the ferric chloride test (Figure 8), were abundant in the adaxial epidermis and hypodermis of young (Figure 9) and senescent leaves. The deposits were situated in similar positions in the SEMs. The cuticle was very pronounced in senescent leaves (Figure 10).

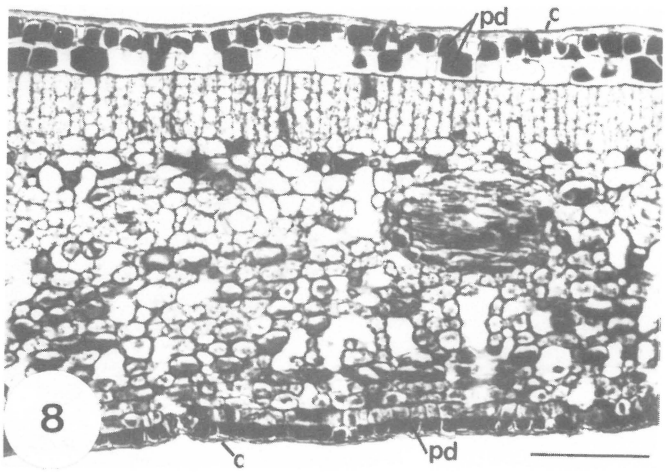
Submerged leaves (litterbags)

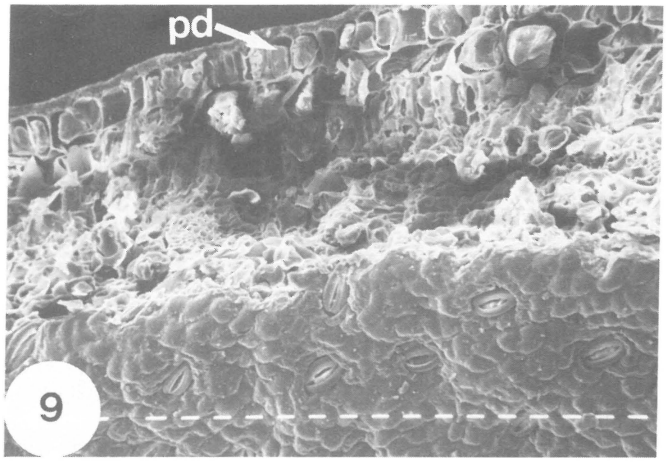
No phenolic deposits were observed in submerged leaves after one week (Figure 11). After three weeks of submergence, fungi had infiltrated the parenchymatous tissue and by the end of week 7 the parenchymatous cells appeared devoid of cellular contents and hyphae were seen within them (Figure 12). Epidermal cells were attacked later and at the end of the study the leaves of *B. gymnorrhiza* were reduced to an assemblage of remnants of the lignified tissue (Figure 13) and the cuticles.

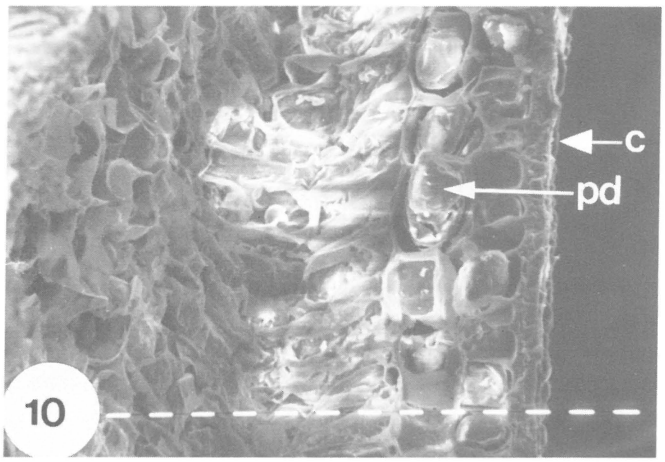
Discussion

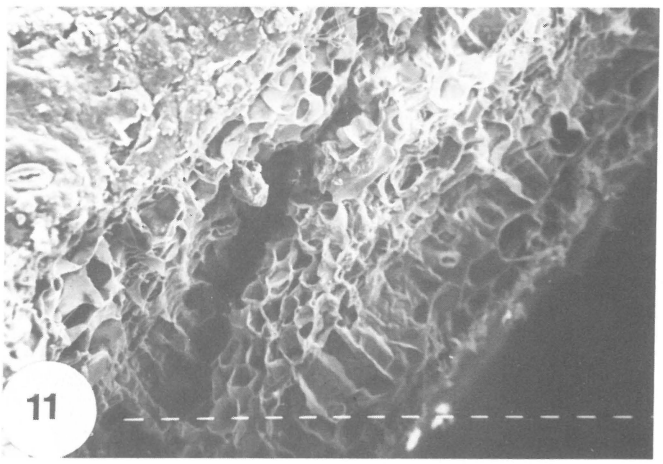
Both saprophytes and parasites are known to occur on the surfaces of green leaves, originally termed the phyllosphere by Last (1955) and now recognized as the phylloplane (Dickinson 1965). In this study no evidence of any biodegradation in the phylloplane was found, supporting the evidence of MacNamara & Dickinson (1981) that, although phylloplane fungi may be cutinolytic or cellulolytic, their activities are not evident in living leaves. Phenolic deposits, which may have had an inhibitory effect on fungal growth, were abundant in pre-abscissed leaves of *B. gymnorrhiza*.

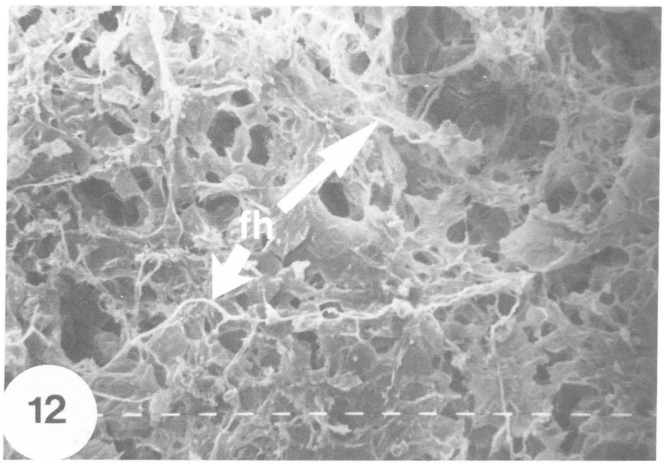
The most discernible feature of the submerged leaves was the presence of cuticular perforations in the adaxial cuticle. These were not observed in senescent leaves prior to submergence. It may be assumed that their formation was initiated upon submergence. The abundance of cuticular

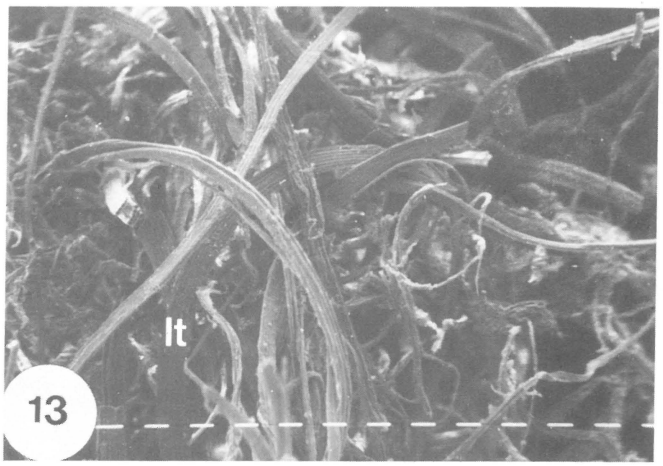












Figures 8 – 13 Light micrograph (Figure 8) and scanning electron micrographs (Figures 9 – 13) of internal features of leaves of *B. gymnorrhiza*. 8. T/S of mature green leaf showing epidermal and hypodermal cells filled with dense phenolic deposits (pd). 9. Young leaf showing phenolic deposits (pd). 10. Yellow senescing leaf showing phenolic deposits (pd) and distinct cuticle (c). 11. No phenolic deposits in leaf after submergence for one week. 12. Fungal hyphae (fh) within leaf tissue after seven weeks of submergence. 13. Completely disintegrated leaf after 18 weeks of submergence showing only the lignified tissue (lt) remnants. Bars = 100 μm in Figure 8 and 10 μm in Figures 9 – 13.

perforations did not appear to increase with decomposition, indicating that they were formed soon after submergence. Steinke *et al.* (1990), however, observed these perforations on the adaxial surface of senescent leaves but found that they enlargened and increased in frequency after a week's submergence. Cutinase activity of fungi has been reported (MacNamara & Dickinson 1981). It is possible therefore that the perforations may have been created by fungal activity thereby giving access to the internal tissues. Cuticular perforations were not present on the abaxial surface, where stomatal pores may have allowed fungal penetration. Cuticular perforations were not observed in decaying leaves of *R. mangle* (Cundell *et al.* 1979).

Cundell *et al.* (1979) and Wilson *et al.* (1986) reported

that soluble phenolics are usually lost rapidly during leaching, which is supported in present findings. Concomitant with loss of these deposits was the extensive growth of fungi, internally and externally, which strongly suggests that phenolics may have had an initial inhibitory effect on their earlier growth.

Chemically, the cuticle consists of two groups of lipid substances – insoluble polymeric cutins which constitute the framework, and soluble waxes deposited on the surface as epicuticular wax and embedded within the cutin matrix as cuticular wax (Holloway 1982). MacNamara & Dickinson (1981) proposed that wax was probably assimilated more rapidly by micro-organisms than cutin. This view is supported by the present study, as progressive microbial wax breakdown on both leaf surfaces occurred throughout the period of decay. Within the leaf, spongy mesophyll cells showed the first signs of decay and degradation of epidermal tissues followed subsequently. Lignified tissues and cuticles were still intact upon termination of study. Over a period of 126 days, fungal colonization of cutin was not observed, indicating its function as a protective barrier.

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